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Soil and starter fertilizer and its effect on yield and protein composition of malting barley

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Abstract

Fertilizer application and growing locations are known to influence yield and protein concentration of malting barley. The aim of the present investigation was to evaluate the influence of soil and starter fertilizer on yield and protein composition in mature and maltedbarley. The cultivar Prestige was grown in two different soils (Lunnarp and LaxmansÅkarp) in combination with the use/non-use of starter fertilizer in climate chambers. Yield parameters, protein concentration and composition was measured. Effect of soil on plant emergence, yield and protein composition was significant while the effect of starter fertilizer was not. More nitrogen rich and low humus content soil (Lunnarp) resulted in higher grain yield and polymerization of proteins and lower protein concentration than the other soil. Combination of soil and starter fertilizer influenced protein composition in mature and malted barley. Breakdown of proteins were significantly higher at certain combination of soil and starter fertilizer than with other combinations. The Lunnarp soil combined with starter fertilizer was preferable to obtain high yield, low protein concentration and large grain size in mature grains. When breakdown of proteins at malting was taken into consideration as well, Lunnarp soil together with no starter fertilizer might be the best option.

Keywords: controlled conditions, *Hordeum vulgare*, location, mature barley, malted barley, optimized nutrients.

Abbreviations

SDS-Sodium dodecyl sulphate; eSMP-SDS-extractable monomeric proteins; uSMP-SDS-unextractable monomeric proteins; TOTE -Total SDS-extractable proteins; TOTU-Total SDS-unextractable proteins; %LUPP-Percentage of large SDS-unextractable polymeric proteins in total large polymeric proteins; %TUPP-Percentage of SDS-unextractable polymeric proteins in total polymeric proteins; Monopol-Monomer/Polymers; SOM-Soil organic matter; DAS-days after sowing.

1. Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop grown worldwide not only for food and feed but it is also used as a raw material for the malting process to produce beer or other alcoholic beverages (Henry, 1989; Celus *et al*., 2006). The profitability of malting barley is influenced largely by the grain yield which in turn depends on a number of factors including differences in cultivar, farming conditions, soil and climate (Fathiet *et al*., 1997). In cool and moist regions early sowing is used for increasing the length of the growing season, thereby leading to increased grain yield (Barber, 1995). However, the early sowing often exposes the young seedlings to low soil temperature (Barber, 1995). Low soil temperature limits the mobility of nutrients in the soil and reduces the root growth, and thereby the availability of nutrients is limited, especially of those transported by diffusion, such as phosphorus (P) and potassium (K) (Barber, 1995). To overcome the nutrient deficiency that is often present at early sowing, the use of starter fertilizers is one option (Kristoffersen *et al*., 2004). Starter fertilizer is considered to enhance seed vigor and early growth, in periods of low nutrient status of soil, limited mobility of nutrients in soil, low soil temperature and slow root growth (Kristoffersenet *et al.,* 2005). Effects of starter fertilizers on yield have primarily been evaluated in vegetables (Masauskas *et al*., 2008) but have also been shown positive for malting barley yield in Finland and Norway (Kleemola *et al*., 1998; Kristoffersen *et al*., 2005). However, excess of starter fertilizer application can also result in salt damage of the roots in the germinating seeds as has been shown e.g. in corn (Bates, 1971). Furthermore, soil initial nutrient levels and soil chemical composition are thought to affect the efficiency of starter fertilizers in barley (Riley, 1983; Kristoffersen . 2005). It has been found that the effect of starter fertilizer, on growth and yield of crop varies with different field locations (Stone, 2000). Influences of certain parameters such as growth and yield are difficult to judge from field experiments due to large environmental variations. Due to the mentioned variations, fertilizer effects on certain plant characters can preferably be studied under controlled conditions. To our knowledge, few studies (Hellgren and Nilsson, 2002) are available investigating the positive effects of starter fertilizer on plant growth and yield of malting barley in controlled conditions.

Both protein concentration and composition play an important role in determining the malt quality (Swanston *et al*., 1995; Wang *et al*., 2007). The protein concentration of malting barley (preferably 9.5-11.5% on dry basis, in most countries of the world) plays a crucial role in determining the quality of the malting barley (Gali and Brown, 2000; Palmer, 2000). Starter fertilizers may help the crop to accumulate more N in the grains, affecting indirectly the grain protein concentration (Masauskas *et al*., 2008). Availability of nutrients, especially N and P, influences the protein and nucleic acid synthesis, and K influences protein synthesis, enzyme activation, osmo-regulation and root growth (Grant *et al*., 2001). It is also suggested that not only the protein concentration but also the protein composition may play an important role in determining the malt quality (Wang *et al*., 2007).

The influence of starter fertilizers on the grain yield, protein concentration and composition of malting barley in controlled conditions has not been evaluated. Furthermore, only few studies have evaluated protein composition in mature barley grains and relations to malt quality (Molina-Cano *et al*., 2002; Wang *et al*., 2007). Studies about changes in protein composition during malting and how this is related to protein composition in mature grainare even scarcer.

Environmental variations, such as growing location and year are well known to influence grain yield and protein concentration in barley (Zhang *et al*., 2001) and also protein composition in wheat (Johansson, 2002). Recent findings have indicated the importance of soil parameters on yield, protein concentration/composition in wheat and barley (Wang *et al*., 2007; Andersson and Holm, 2011). Influences on grain yield and protein parameters by soil and soil-starter fertilizer interactions have been limitedly investigated both under field and controlled conditions.

Therefore, the aim of the present investigation was to evaluate the effect of soil and starter fertilizer on growth, yield, protein concentration and composition of malting barley, in controlled conditions. Further, the aim was to investigate whether protein concentration and composition in mature barley grains influenced protein concentration and composition in malted barley grains.

2. Materials and methods

The spring malting barley cultivar Prestige with a germination rate of 96% was used. The plants were grown in controlled climatic chambers in the Biotron (manufactured by ÖKG-Grünbach, Austria and Skanska, Sweden) at the Swedish University of Agricultural Sciences (Alnarp, Sweden). A light intensity of 500 μmol $m^{-1}s^{-1}$ was provided by fluorescent tubes (Sylvania Lumiance, type F 96 T12 VHO cold white 215W, Osram Sylvania, Mississauga, ON, Canada) installed in the ceiling of the climate chamber. Settings of weekly day and night lengths (taken from the calendar of sunrise and sunset), weekly average day and night temperatures and relative humidity in the climatic chamber are shown in Table 1. The temperature (°C) settings in the Biotron were calculated as weekly means of the period April 10th to August 10th from a series of measurements of seven years (1987-1993) logged climate data from the small village Ädelholm (55.66°N and 13.19°E), just outside Staffanstorp (Sweden), close to the sites where the soils were taken and where Nordic Sugar mill has an experimental site with a weather station.

Table 1. Climate used in the climate chambers when growing the malting barley cultivar Prestige.

Week	Sunrise	Sunset	MNT	MDT	RH
1	04:40	19:20	5	9	80
2	04:20	19:30	5	9	80
3	04:00	20:20	8	12	80
4	03:40	20:30	10	13	80
5	03:20	20:50	11	15	80
6	03:00	21:00	12	15	80
7	02:50	21:20	13	17	80
8	02:30	21:40	13	15	80

Week	Sunrise	Sunset	MNT	MDT	RH
9	02:30	21:40	14	18	80
10	02:20	22:00	14	17	80
11	02:20	22:00	15	18	70
12	02:20	21:50	16	19	70
13	02:30	21:50	17	20	70
14	02:40	21:40	15	18	70
15	02:50	21:30	16	28	70
16	03:10	21:10	16	19	70
17	03:30	21:00	16	19	70
18	03:40	20:50	16	19	70

MNT=Mean night temperature, MDT=Mean day temperature (°C), RH= relative humidity (%).

2.1 Soil preparation

In order to resemble field conditions, large containers, with a size of 80 cm x 60 cm x 40 cm, were used in the experiment. The top soil used for growing the plant material in the containers was taken from two locations i.e. LaxmansÅkarp (55.73°N and 13.10°E) and Lunnarp (55.54°N and 14.04° E). The two soils were selected from two representative fields where malting barley of good quality have previously been grown. The soil from both the locations was heated in the oven for 20 h at 105°C in order to kill all the pathogens and microorganisms that could affect the plant growth and development. The detailed information about the soil texture and content is given in Table 2. After oven drying, the soil was sieved (2*2 cm) and blended to remove structure differences. In the bottom of every container 10 cm of perlite was placed as a water buffer. Above the perlite layer, 25 cm of soil was placed. Due to oven drying, all the water in the soils was removed. Throughout the experiment, the soil in each container was kept at a suitable humidity for the crop to avoid water stress. To ensure enough humidity in the soils, one of the containers in each climate chamber was placed on a weighing balance, by which the amount of water loss in the containers was assessed. This loss in amount of water was added to all the containers n this climate chamber every second or third day (depending on how much water was lost) with a bucket having a sprinkler tap on it. Thus, the same amount of water was added to both types of soil and to both treatments.

Table 2. The amount and percentage of different nutrients and soil texture of the used soils from LaxmanÅkarp and Lunnarp before and after drying.

Soil from different locations				
	Laxmans Åkarp		Lunnarp	
mg $100g^{-1}$	BD	AD	BD	AD
P-Al	10.1	12.1	3.90	4.80
K-Al	5.90	6.70	10.1	12.5
Mg-Al	8.90	7.30	15.5	13.7
K/Mg	0.70	0.90	0.60	0.90
Ca-Al	531	543	370	396
Humus Content (%)	4.00	4.10	3.10	3.10
Clay Content (%)	14.0	12.0	22.0	23.0

Soil from different locations				
	Laxmans Åkarp		Lunnarp	
Silt (%)	33.0	33.0	35.0	34.2
Sand (%)	49.0	50.9	39.9	39.7
NH_4-N (kg ha^{-1})	14.4	13.9	14.0	27.4
NO_3-N (kg ha^{-1})	14.1	11.6	7.60	4.80
N-MIN (kg ha^{-1})	28.5	25.5	21.6	32.2

BD= before drying, AD= after drying.

2.2 Replicates, treatments, sowing and fertilizer management

The description about each treatment regarding soil and fertilizer management is given in Table 3. Two climate chambers and eight containers were used in the experiment, four containers in each chamber. Each climate chamber was considered as one replicate of the experiment. In each chamber, four different treatments were applied, one in each of the four containers. In treatment one and two, soil from LaxmansÅkarp was used. In the treatment three and four, soil from Lunnarp was used. The placement of the containers in the chambers was randomized. Hand sowing was done in all treatments by using a seed rate of 365 growing seeds m^{-2}. After sowing, starter fertilizer (8.016 g $container^{-1}$) was given to treatment one and three having soils from LaxmansÅkarp and Lunnarp, respectively. Park Complete (N-P-K: 12-5-15 manufactured by Yara AB, Landskrona, Sweden) was used starter fertilizer. After application of starter fertilizer in the seed row, a 2 cm layer of soil was spread by hand for making seedbed. The basal dose of N (100kg $hectare^{-1}$) was applied to all the four treatments including the ones which were given starter fertilizer. For basal dose of N, the source used was Yara Mila (N-P-K: 24-4-5) manufactured by Yara AB, Landskrona, Sweden. After broad casting of the basal fertilizer application, a very thin layer (1 cm) of silver sand (Baskarps sand) was placed on the top layer of soil. The sand not only serves as an indicator of the water status of the soil but also for leveling out eventual evaporation differences.

Table 3. Description of treatments used for the growing of the spring malting barley cultivar Prestige in climate chambers.

Treatment	Soil location	Starter fertilizer
1	Laxmans Åkarp	Applied
2	Laxmans Åkarp	Not applied
3	Lunnarp	Applied
4	Lunnarp	Not applied

2.3 Harvesting and storage

The spikes were harvested when the plants reached maturity at the Zadoks decimal code 92 (Zadoks *et al*., 1974). Grains from each treatment were weighed, lyophilized and weighed again, and thereafter grain water content was calculated. For breaking the dormancy of seeds in order to facilitate the malting, the grains were stored in a seed storage room for three months at 2°C. Grain samples from the mature barley grains as well as from malted barley grains were milled in a Yellow Line commercial Blender (A 10, IKA- Werke, Staufen, Germany) for determination of amount and size distribution of polymeric proteins (ASPP) (Johansson *et al*., 2005; Malik *et al*., 2011).

2.4. Sampling and measurements of parameters related to plant growth and possible final yield

Plant emergence was measured by counting the number of emerged seedlings in each container, at 14, 17, 20, 24 days after sowing (DAS) and finally at full emergence (30 DAS). For measuring of the plant height, number of tillers and length of top first, second and third leaf, fifteen plants were used in each container. To make the measurements as random as possible every tenth plant was selected. Plant height was measured with a measuring tape, from the soil to the top of the plant. After threshing, the grain yield (g) and thousand kernel weight (g) for each combination of treatment was measured. Protein concentration (%) (measured by NIR at Agrilab AB, Uppsala, Sweden), grain size > 2.5 mm (%) and grain bulk density (g L^{-1}) of each treatment was also measured.

2.5 Malting

Malting was carried out in the micro-malting plant at SLU, Alnarp, Sweden. This apparatus was designed by Danbrew consult Ltd, Copenhagen V, Denmark. Seed samples from each of the treatments and replicates together with one standard sample were used for malting. The weight of each sample was 80 g. The malting was done according to Henry and McLean (1984) with some modification with the following three main steps. The samples were steeped for 6 h by immersion, followed by 11 h of dry period, with humidity maintained by passing air through a fine water spray. Temperature was maintained at 14.5°C. By the end of steeping all the grains were fully hydrated and showing initial signs of germination - the formation of a tiny rootlet or "chit". Germination continued after the steeping for a further 4-5 days (96-120 h) with grain moisture maintained at 44-45%. Temperature was maintained at 14.5°C. The temperature in the kiln was increased uniformly from 40°C to 60°C over 11 h and held at 60°C for 2 h. The temperature was then increased uniformly from 60°C to 80°C over 4 h and held at 80°C for a further 4 h. The temperature was allowed to fall to 40°C over the next 1.5 h. After a further 43 h the malted grains was reheated up till 60°C, just before the rootlets were removed. The rootlets were removed manually by rubbing the seeds together with the hand in a plastic bag.

2.6. Endosperm modification

To check how much proteins have been modified in the endosperm during malting, the determination of endosperm modification was carried out according to Henry (1989). The endosperm modification was carried out before the kilning process was initialized.

2.7. Protein analysis

In a number of investigations, protein composition has been evaluated by the use of size exclusion high pressure liquid chromatography- SE-HPLC (Johansson *et al.,* 2005, 2008). As we were interested to evaluate protein composition in malting barley and how the malting process influence the protein composition, SE-HPLC was used with a two step extraction procedure according to Johansson *et al.* (2005) and Gupta *et al.* (1993). In the first step the SDS-soluble protein fractions (SDS-extractable proteins) were extracted while in the second step SDS-insoluble protein fractions (SDS-unextractable proteins) were extracted using sonication. Proteins were separated by SE-HPLC and detected at 210 nm. SE-HPLC analyses was performed on a Waters (Milford, MA, USA) HPLC system using a BIOSEP SEC-4000 Phenomenex column. For the SE-HPLC analyses three replicates were analyzed from each container i.e. of each sample and replicate. The total area under the chromatogram was used to

calculate TOTE= total SDS-extractable proteins and TOTU= total SDS-unextractable proteins. The SE-HPLC chromatograms of both SDS-extractable and SDS-unextractable proteins were divided into two main parts which were further subdivided into two sections (Figure 1). The first part of the chromatogram representing polymeric proteins (PP), with a retention time interval 9.5-17 minutes, was divided into large PP (LPP) and small PP (SPP) (Malik *et al.,* 2011). The second part of chromatogram corresponding to monomeric proteins (MP), with a retention time interval 17-29 minutes, was divided into large MP (LMP) and small MP (SMP). The amounts of SDS-extractable (e) and unextractable (u) small monomeric proteins (eSMP and uSMP) were measured according to Johansson *et al.* (2005). Percentage of TUPP [SDS-unextractable PP/total (SDS-unextractable + SDS-extractable) PP x 100] was calculated according to Gupta *et al*. (1993). The PP part of the chromatogram containing the largest PP (L) was used to calculate %LUPP according to Johansson *et al*. (2005). Monopol (Monomers/Polymers) [(SDS-extractable MP+SDS-unextractable MP)/(SDS-extractable PP+SDS-unextractable PP)] was calculated according to Johansson *et al.* (2008).

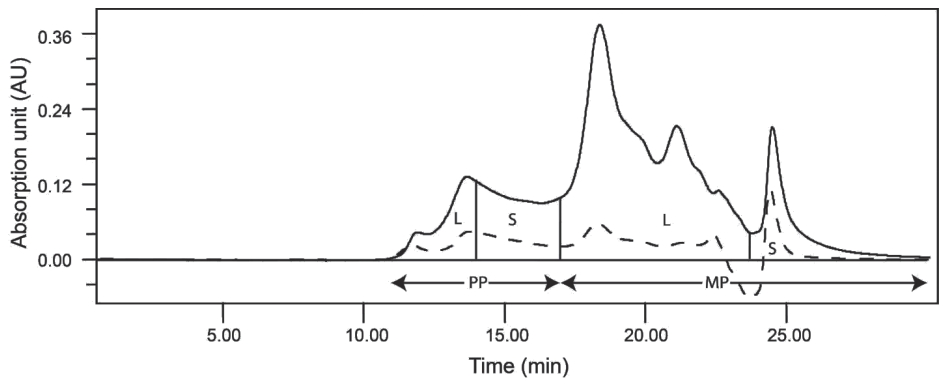

Figure 1. SE-HPLC chromatogram of SDS-extractable proteins (___) and SDS-unextractable proteins (---), respectively. The chromatogram was divided into two main parts comprising of polymeric proteins (PP) and monomeric proteins (MP), respectively. Each main part of the chromatograms was subdivided into two parts [designated as Large (L) and small (S)].

2.8. Relative content of protein fraction

For each of the protein fractions and treatments (for description see Table 3) the content was set to 100% in the mature grain and thereafter the relative content was calculated in the malted barley grains. Thus, a value above 100 indicates an increase while a value below 100 indicates a decrease of the protein fraction by the malting process.

2.9. Statistical analysis

MS Excel and the statistical package SAS (SAS, 2004) were used for figures and data analysis, respectively. Data evaluation was done by the Spearman rank correlation and analysis of variance procedures using SAS.

3. Results

3.1 Effect of treatments on plant growth

Significantly higher number of plants had emerged, at 20 DAS, in soil from LaxmanÅkarp in comparison with soil from Lunnarp (Table 4). A significantly higher number of plants had emerged at 14 DAS when no starter fertilizer was applied as related to when starter fertilizer was applied (Table 4). However, the final number of plants at full emergence (30 DAS) was not influenced significantly, neither by the used soil nor by applied/not applied starter fertilizer dosage (Table 4). The treatments (combination of soil from different locations and applied/not applied starter fertilizer) did not significantly influence the number of emerged barley plants at different DAS (Table 4). Anthesis and maturity date did not differ significantly among treatments (results not shown).

Table 4. Mean values of plant emergence, length of the leaves and plant height at starter fertilizer dosage, two soil locations and four different treatments (for description see Table 3).

	Emerged plants						
	14 DAS	20 DAS	30 DAS	1st leaf (cm)	2nd leaf (cm)	3rd leaf (cm)	Plant height (cm)
	Starter Fertilizer						
Applied	72.2b	160.8a	163.2a	36.7a	28.7a	20.1a	61.3a
Not applied	111.0a	162.0a	162.2a	35.2a	27.6a	19.2a	62.3a
Soil locations							
Laxmans Åkarp	91.2a	163.0a	164.2a	37.3a	29.3a	20.5a	59.1b
Lunnarp	92.0a	159.8b	161.2a	34.6b	27.0b	18.9a	64.5a
Treatments							
Treatment 1	63.0a	162.0a	164.0a	39.0a	30.5a	21.4a	58.3b
Treatment 2	119.5a	164.0a	164.5a	35.6b	28.1ab	19.5ab	59.9b
Treatment 3	81.5a	159.5a	162.5a	34.4b	27.0b	18.9b	64.3b
Treatment 4	102.5a	160.0a	160.0a	34.8b	27.0b	18.9b	64.8a

Means with the same letter within a column are not significantly different (LSD method <0.05). DAS= Days after sowing.

The soil from LaxmansÅkarp resulted in significantly longer first and second leaves of the barley as compared to when soil from Lunnarp was used (Table 4). No significant differences were seen in the length of the third leaf when soils from different locations were used (Table 4). Applied/not applied starter fertilizer dosage did not influence the length of the first, second or third leaf significantly (Table 4). The first leaf from the top of the plant was significantly longer in treatment one (soil from LaxmansÅkarp in combination with the application of starter fertilizer) in comparison with the other treatments (Table 4). Also, the second and third leaf from the top was long in treatment one although

statistically similar with treatment two (soil from LaxmansÅkarp in combination with no application of starter fertilizer) in comparison with the other treatments (Table 4).

Plant height was not significantly affected by the applied/not applied starter fertilizer dosage (Table 4). Number of tillers per plant was not significantly influenced by the origin of soils,application of starter fertilizer dosage or treatments (results not shown). The significantly highest plants were obtained from treatment four (soil from Lunnarp in combination with no application of starter fertilizer) as related to when soil from LaxmanÅkarp and other treatments were used (Table 4).

3.2 Effect of treatments on yield

Soil originating from different locations affected the yield significantly (Table 5). The soil from Lunnarp resulted in significantly higher yield as compared to the soil from LaxmansÅkarp (Table 5). Applied/not applied starter fertilizer dosage did not significantly influence the grain yield (Table 5). Grain yield was not significantly influenced by the different treatments (Table 5).

3.3 Effect of treatments on quality parameters

Grains from plants grown on soil from LaxmansÅkarp were having higher protein concentration as compared to those grown on soil from Lunnarp (Table 5). Applied/not applied starter fertilizer dosage did not influence significantly the barley grain protein concentration at maturity (Table 5). Significantly the highest grain protein concentration were found, at maturity, when treatment one (soil from LaxmansÅkarp in combination with the application of starter fertilizer) was used in comparison with the other treatments (Table 5).

Table 5. Mean values of yield and quality parameters at maturity of spring malting barley at starter fertilizer dosage, two soil locations and four different treatments (for description see Table 3).

	Yield ($g\ m^{-2}$)	**Protein concentration (%)**	**Grain size >2.5 mm (%)**
Starter Fertilizer			
Applied	343.2a	11.1a	97.8a
Not applied	354.6a	10.7a	98.6a
Soil locations			
Laxmans Åkarp	311.9b	11.3a	96.8b
Lunnarp	385.8a	10.5b	99.6a
Treatments			
Treatment 1	296.9a	11.7a	96.0b
Treatment 2	326.9a	10.9b	97.6ab
Treatment 3	389.5a	10.5b	99.6a
Treatment 4	382.2a	10.5b	99.6a

Means with the same letter within a column are not significantly different (LSD method <0.05).

Grains from the plants grown on soil from Lunnarp had significantly higher % of grains with a grain size > 2.5 mm as compared to those grown on soil from LaxmansÅkarp (Table 5). Applied/not applied starter fertilizer dosage did not significantly influence the % of grains with a grain size > 2.5 mm at maturity (Table 5). The % of grains with a grain size > 2.5 mm was significantly lower in grains from treatment one (soil from LaxmansÅkarp in combination with the application of starter fertilizer) as compared to those from treatment three and four (soil from Lunnarp combined with and without the application of starter fertilizer) (Table 5). The grain bulk density was not influenced by applied/not applied starter fertilizer, soil from different locations or the treatments (result not shown).

3.4 Effect of treatments and malting process on protein composition and content.

In mature barley grains, the soil from Lunnarp resulted in significantly higher %LUPP and Monopol as related to the soil from LaxmansÅkarp (Table 6). Starter fertilizer application did not significantly influence the protein composition in the mature barley grains (Table 6). In mature grains of barley the amounts of TOTE were significantly higher in treatment one (soil from LaxmansÅkarp in combination with the application of starter fertilizer) as compared to treatments two and three (Table 6). Also, %LUPP were significantly lower in treatment one as compared to treatment three and four and Monopol was significantly lower in treatment one as compared to the other treatments (Table 6).

Table 6. Mean values of the protein fractions in mature barley grains of the barley cultivar Prestige grown at starter fertilizer dosage, two soil from different locations and four different treatments (for description see Table 3).

Source	eSMP	uSMP	TOTE	TOTU	% LUPP	% TUPP	Monpol
Starter Fertilizer							
Applied	12.5a	3.98a	101a	25.8a	39.8a	32.2a	2.73a
Not applied	12.4a	4.08a	97.6a	25.7a	42.2a	33.5a	2.78a
Soil locations							
Laxmans Åkarp	12.2a	4.06a	101a	26.7a	38.0b	31.5a	2.66b
Lunnarp	12.6a	4.00a	97.3a	24.8a	44.0a	34.2a	2.85a
Treatments							
Treatment 1	12.4a	3.93a	107a	26.7a	35.2b	29.6a	2.59c
Treatment 2	12.1a	4.19a	95.2b	26.0 a	40.7ab	33.4a	2.73b
Treatment 3	12.6a	4.03a	94.5b	24.9a	44.4a	34.7a	2.87a
Treatment 4	12.6a	3.96a	100ab	24.7a	43.6a	33.7a	2.82ab

Means with the same letter within a column are not significantly different (LSD method <0.05).

Differences in the amount of changes in the protein composition, during malting process, were found for the different treatments (Table 7). Treatment two (soil from LaxmansÅkarp combined with no application of starter fertilizer) increased significantly more the amount of eSMP as compared to treatment three (soil from Lunnarp combined with starter fertilizer), during malting process (Table 7). The amounts of TOTU, %LUPP and %TUPP decreased more in treatment two (soil from LaxmansÅkarp combined with no application of starter fertilizer) in comparison with the treatment one (soil from LaxmansÅkarp in combination with the application of starter fertilizer), during the malting process (Table 7).

Table 7. Relative content of protein fractions after the malting process.

	eSMP	uSMP	TOTE	TOTU	%LUPP	%TUPP	Monopol
Starter fertilizer							
Applied	112b	167a	121a	102ab	58b	84a	161ab
Not applied	117ab	169a	124a	98.1ab	52b	76ab	162ab
Soil locations							
LaxmansÅkarp	118ab	168a	123a	102ab	59b	82a	177a
Lunnarp	113ab	168a	122a	101ab	51b	77ab	147b
Treatments							
Treatment 1	114ab	171a	119a	106a	81a	85a	178a
Treatment 2	121a	166a	128a	91.0b	60b	63b	176a
Treatment 3	110b	163a	124a	97.0ab	58b	63b	145b
Treatment 4	114ab	174a	120a	106a	63ab	69ab	149b

Means with the same letter within a column are not significantly different (LSD method <0.05).

The amounts of TOTE in mature barley grains were negatively correlated to uSMP in malted barley grains (Table 8). Also, a positive correlation was seen between TOTE, in mature barley grains, and Monopol, in malted barley grains (Table 8). Percentage of %LUPP and %TUPP in mature barley grains were positively correlated with uSMP in malted barley grains (Table 8).

Table 8. Spearman rank correlations coefficients while comparing amount of different protein fractions (fract.) in mature and malted barley grains (N=24) of spring malting barley cultivar Prestige.

	Protein fract. of malted barley grains				
Protein fract. of mature barley grains	eSMP	uSMP	%LUPP	%TUPP	Monopol
TOTE	0.07	-0.54**	0.36	0.34	0.45*
TOTU	-0.15	0.38	-0.18	-0.19	-0.20
%LUPP	-0.20	0.57***	-0.47*	-0.45*	-0.43*
%TUPP	-0.22	0.59***	-0.42*	-0.40	-0.49*

*, **, ***= Significant at p<0.05, 0.01, 0.005.

4. Discussions

In the present investigation soil originated from different locations was of major importance for emergence, yield and protein composition of malting barley. Also, combination of treatments i.e. soil from different locations combined with differences in application of starter fertilizer, affected plant growth, yield and protein composition and concentration in the present investigation. However, in contrary to earlier investigations on vegetables (Stone, 2000) and barley (Hellgren and Nilsson, 2002; Kristoffersen *et al.,* 2005), the use of starter fertilizer did not show a general and independent influence on plant growth, development and protein composition in barley seeds in the present investigation.

As can be seen from Table 2, the two soils used in our study differed in a range of parameters. One obvious difference between the two soils were also their reaction to the drying procedure, resulting in an increase content of NH_4-N and N-MIN together with a decrease in content of NO_3-N in the soil from Lunnarp while the N contents of soil from LaxmansÅkarp remained rather stable. The total nitrogen content in the soil from Lunnarp was thereby higher after drying than the content in the soil from LaxmansÅkarp which might be part of explanation for the higher yield in malting barley grown on the soil from Lunnarp. However, the differences in N content between the two soils was around 7 kg ha^{-1} (Table 2), which is a negligible sum, especially as a basal dose of N of 100 kg ha^{-1} was applied. The soil from the location that resulted in high grain yield (Lunnarp) was lower in soil organic matter (SOM) or humus content than soil from the other location (LaxmansÅkarp). Also, the soil from the Lunnarp location had higher clay (%) and silt (%) content than the soil from LaxmansÅkarp. SOM is important for macro and micronutrients supply as well as for moisture availability and buffer capacity. It also affects soil aggregate stability and soil structure in general (Riley, 1983). Thus, the variation between the two soils in SOM and clay/sand might be the main reason explaining differences in yield of malting barley in the present experiment. One might speculate that especially during field conditions, higher SOM and clay content might result in higher moisture availability and buffer holding capacity. However, after the pre-treatment of the soils and the equal additions of water and avoidance of drought in the soils, variation in moisture availability between the two used soils seems less likely. Another explanation might be variation between the two soils in amount of nutrients available at various times during crop development. It is well known that early N availability increase biomass of the plant at early development stages which is also related to increased yield (Malik, 2012). Also, N availability has been found an important parameter for protein composition in wheat (Johansson *et al*., 2005). However, impact of various parameters is related to the temperature during crop cultivation (Malik *et al*., 2013). To be able to fully explain the impact of various soil parameters on yield and protein composition, additional screening of soils, soil parameters, yield and protein factors are needed.

Thus, starter fertilizer seemed to influence the results more when applied to the soil from LaxmanÅkarp, than when applied to the soil from Lunnarp. TOTE is known to correlate positively with protein concentration in wheat (Johansson *et al.,* 2003). If the amount of TOTE is related to high protein concentration also in malting barley, high amounts might lead to a decrease in malt quality (Palmer, 2000). Therefore, soil initial fertility level should be considered in scheduling the fertilizer dosage for malting barley production. This consideration of fertilizer management may help in attaining desired protein concentration for beer production (Gali and Brown, 2000). Soil from Lunnarp in combination, with starter fertilizerand without starter fertilizer, has resulted in grains with the highest amount of %LUPP and Monopol. In wheat, a short grain filling period both determined genetically (Malik *et al.,* 2011) and environmentally (Johansson *et al.,* 2005; 2008) has been shown to correlate to a high %LUPP (Large UPP). If the differences in treatments have resulted in various times for grain filling period, was not investigated in this study.

The most relevant finding from the present investigation was that the choice of soil and the use of starter fertilizer have influenced the breakdown patterns of proteins during malting. Thus, the effect of treatments seems more remarkable for protein composition after malting than in mature barley. Generally, high protein concentration in the mature barley grain (TOTE; Treatment 1 and 4), resulted in high breakdown rate of easily extractable proteins, resulting in a low decrease in %LUPP and %TUPP and an increase in TOTE and/or TOTU, as was also described in Malik (2012). The importance of not just investigating protein composition in mature grains but also during processing, has been emphasized recently also in wheat during mixing, where protein composition was not straightly correlated with protein composition in the grain (Hussain *et al*., 2012). Variation in breakdown of proteins depending on treatment during cultivation has not been reported previously, to our knowledge. One might speculate that a higher breakdown rate will result in higher amounts of free amino acids and peptides within the malted barley. The smaller protein related molecules can easily be transferred into the ready beer and influence the beer quality. These issues need more investigations before final conclusion can be drawn as to how cultivation practices influence the beer quality on a protein related molecular scale.

The small protein molecules found in the SMP fractions besides peptides and amino acids are albumins (Johansson *et al.,* 2008). Thus barley albumins such as z-proteins and lipid transfer proteins (LTP) are supposed to be found in the SMP fraction. Z-proteins are considered good for foam stability and LTP are considered important for foam formation (Sørensen *et al.,* 1993). In the present investigation, no differences in SMP were found, in mature barley grains, among the used treatments. More investigations are needed, especially of the SMP fractions, in order to understand how protein composition is influencing beer quality.

5. Conclusions

Soil from different locations was found to play a major role in influencing the early growth stages and yield of spring malting barley. In the present study, the soil N-content was positively related to yield and polymerization of the proteins, while negatively related to protein concentration. In certain soils, starter fertilizer can contribute to establish the later growth (plant height, leaf length) of a crop, but also to increase grain protein concentration. For achieving high yields, desired protein concentration and composition, combination of specific soil location with starter fertilizer are of utmost importance. In the present investigation, treatment 3 (soil from Lunnarp with starter fertilizer application) seemed preferable for mature barley with high yield, low protein concentration, large grain size and low TOTE. However, as large breakdown of proteins at maturity might be negative, treatment 4(soil from Lunnarp with no starter fertilizer application) might be more desirable.

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